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Determination of Creatinine in Human Serum by Isotope Dilution-Mass Spectrometry

Definitive Methods in Clinical Chemistry, IV

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Summary: A method for the determination of creatinine in human serum by isotope dilution-mass spectrometry is described. The analytical procedure comprises the following steps:

(1) Addition of [^{13}C,$^{15}N_2$]creatinine to the serum sample;
(2) ion exchange chromatography on the cation exchange resin AG 50W-X2;
(3) formation of the trimethylsilyl derivative;
(4) gas liquid chromatography-mass spectrometry (GC-MS); selected ion monitoring (SIM) at the m/z-values 329 and 332;
(5) calculation of the amount of creatinine in the serum sample from the isotope ratio, as measured by GC-MS.

[^{13}C,$^{15}N_2$]Creatinine was prepared by chemical synthesis. The substance is then used as internal standard for the measurement of creatinine in serum samples.

The imprecision of the method was in the range from 0.35 to 1.05% (coefficient of variation) as determined by repetitive measurements of creatinine in 13 different control sera on different occasions. The lower limit of detection of the mass spectrometer in the selected ion monitoring mode is about 0.5 ng creatinine with a signal to noise ratio of 3 : 1

The accuracy of the method is achieved by the use of the isotope dilution principle in combination with GC-MS. In view of the high specificity and exact control of recovery, the procedure for the measurement of creatinine in human serum, as described here, may be considered as a definitive method in clinical chemistry.

Bestimmung von Kreatinin im menschlichen Serum mit Hilfe der massenspektrometrischen Isotopenverdünnungsanalyse

Definitive Methoden in der Klinischen Chemie, IV

Zusammenfassung: Es wird eine massenspektrometrische Isotopenverdünnungsmethode zur Bestimmung von Kreatinin in menschlichem Serum beschrieben. Das Analysenverfahren umfaßt folgende Schritte:

(1) Zugabe von [^{13}C,$^{15}N_2$]Kreatinin zu der Serumprobe;
(2) Ionenaustauschchromatographie an dem Kationenaustauscher AG 50W-X2;
(3) Bildung des Trimethylsilylderivates von Kreatinin;
(4) Gaschromatographie-Massenspektrometrie (GC-MS), massenspezifische Detektion bei den m/z-Werten 329 und 332;
(5) Berechnung der Menge an Kreatinin in der Serumprobe aus dem massenspektrometrisch ermittelten Isotopenverhältnis.

[^{13}C,$^{15}N_2$]Kreatinin wurde zunächst durch chemische Synthese hergestellt. Die Substanz wurde dann als interner Standard zur Messung von Kreatinin in Serumproben verwendet.

Die Impräzision der Methode lag im Bereich von 0,35 bis 1,05% (Variationskoeffizient); dies wurde durch Mehrfachanalyse von 13 verschiedenen Kontrollseren an verschiedenen Tagen ermittelt. Die untere Nachweisgrenze des Massenspektrometers bei der massenspezifischen Detektion liegt bei etwa 0,5 ng Kreatinin bei einem Signal-zu-Rausch-Verhältnis von 3 : 1.

Die Richtigkeit der Methode wird durch die Anwendung des Isotopenverdünnungsprinzips in Kombination mit der Gaschromatographie-Massenspektrometrie erreicht. In Anbetracht der hohen Spezifität und der exakten Kontrolle der Wiederfindung kann das hier beschriebene Verfahren zur Bestimmung von Kreatinin in menschlichem Serum als definitive Methode in der Klinischen Chemie angesehen werden.

Introduction

The concentration of creatinine in human serum is one of the most frequently determined clinical chemical parameters. The increase of this endproduct of metabolism in blood directly reflects the degree of insufficiency of glomerular filtration in the kidneys. For a long time the only technique for the measurement of creatinine in human body fluids was the colour reaction with picric acid (1, 2). Since this reaction is known to be sensitive to interference from many drugs, additional steps of chromatographic prepurification have been introduced into the analytical procedure (3, 4, 5). During recent years enzymatic reactions (6–9), high performance liquid chromatography (10–17) and GC-MS (18–20) have been proposed for the measurement of creatinine.

Significant differences are observed when the results obtained with various routine methods for the same serum are compared (21). This is also apparent from the results of collaborative surveys of many quality control organisations. Since, at present, there exist no reliable criteria to decide what method would yield the most accurate results, it is necessary to apply several method-dependent target values for the evaluation of the collaborative surveys. In order to overcome this unsatisfactory situation it appeared necessary to develop a reference and definitive method technology for the measurement of creatinine. In the present investigation a definitive method for the determination of creatinine in human serum is described which is based on the principle of istope dilution-mass spectrometry (ID-MS).

Principle of the Method

Serum samples, containing unknown amounts of creatinine, are equilibrated with definite amounts of isotopically labelled [^{13}C,$^{15}N_2$]creatinine. The substances are then isolated from the biological material by the use of an ion exchange resin and converted to the trimethylsilyl derivatives. The ratio of non-labelled to labelled creatinine is measured by combined gas chromatography-mass spectrometry. The isotope ratios, measured after processing the serum samples, are compared with those obtained from standards containing known amounts of labelled and non-labelled creatinine. The unknown amounts of creatinine are calculated from the isotope ratios determined in the serum samples and standards.

Material and Procedure

Reagents

Creatinine (purity 99.8%) is a standard reference material (SRM 914, National Bureau of Standards, Washington, USA).

[^{13}C,$^{15}N_2$]Creatinine is prepared by chemical synthesis as described below. [^{13}C,$^{15}N_2$]Cyanamide (91.6 atom % ^{13}C; 99.5 atom % ^{15}N) is obtained from Amersham Buchler, Braunschweig, FRG. Sarcosine ethyl ester hydrochloride is a product of Fluka, Basel, Switzerland. Tris[hydroxymethyl] aminomethane is supplied by Merck, Darmstadt, FRG. Ion exchange resin, AG 50W-X2, 200–400 mesh, hydrogen form, is obtained from Biorad, München, FRG. The resin is washed with 2 mol/l ammonia und distilled water. The material is then filled into small glass columns (200 × 4 mm) to a height of 4 cm. N-Methyl-N-trimethylsilyl-trifluoroacetic amide (MSTFA) is supplied by Macherey & Nagel, Düren, FRG. Dried pyridine (Merck, Darmstadt, FRG) is stored over molecular sieve 4Å (Merck). All solvents are of analytical grade or distilled prior to use.

Synthesis of [^{13}C,$^{15}N_2$]Creatinine

The preparation of labelled creatinine is based on a chemical reaction which has been described by *Abderhalden & Sickel* (22). 42 mg [^{13}C,$^{15}N_2$]Cyanamide (dried in a desiccator) is dissolved in 160 µl triethylamine which was stored over molecular sieve 4Å. 160 mg Sarcosine ethyl ester hydrochloride (dried in a desiccator) is added. The mixture is stirred with a magnetic agitator overnight at 80°C. The triethylamine is evaporated in a stream of nitrogen at 60°C and the residue is dried under reduced pressure. The crude reaction product is dissolved in 1 ml 0.05 mol/l ammonia and chromatographed on an RP-8 column (25 × 2.5 cm, Lobar, 40–63 µm, Merck, Darmstadt, FRG) with 0.05 mol/l ammonia as eluant. The isotopically labelled creatinine is eluted after about 80 ml. The fractions containing the labelled substance are collected and the solvent is evaporated under reduced pressure. Further purification is achieved by preparative high performance liquid chromatography (HPLC). The labelled creatinine is dissolved and chromatographed in a 0.01 mol/l tris[hydroxymethyl]

aminomethane/hydrochloric acid buffer (pH 7.6). The eluant is pumped at 2 ml/min through the column. The creatinine-containing fractions are collected and the solvent is removed under reduced pressure. Final purification is carried out by recrystallisation from ethanol at 0°C. 41 mg of [^{13}C,$^{15}N_2$] creatinine corresponding to 36% of the theoretical yield is obtained as final product. The purity of the substance was checked by glass capillary column gas chromatography of the trimethylsilyl derivative on a 30 m fused silica column coated with SE-52. The identity of the substance was verified by combined gas chromatography-mass spectrometry (GC-MS) of the trimethylsilyl derivative, which gave the mass spectrum shown in figure 1.

Glassware

For the preparation of the creatinine standard solution a 100 ml volumetric flask is calibrated by filling it to the calibration mark with water at 20°C and weighing the contents. For the sampling of serum as well as for dispensing the non-labelled standard solution an electronically automated pipettor (Microlab P, Hamilton, Bonaduz, Switzerland) equipped with a gas-tight 2.5 ml syringe is calibrated by weighing definite amounts of water at 20°C. For pipetting of the [^{13}C,$^{15}N_2$] creatinine solution a 50 µl-syringe (SGE, Melbourne, Australia) equipped with a repeating adaptor which is adjusted to 25 µl is used. The precision of the pipetting procedure using the syringe and the Hamilton pipettor is determined by weighing appropriate samples of water ten times at 20°C. The water samples are weighed in small plastic vials which are closed immediately after dosage in order to avoid any loss of the water due to evaporation during the weighing procedure. The imprecision of the Hamilton pipettor is about 0.03% (coefficient of variation, CV) and that of the 50 µl-syringe about 0.15%.

Weighing Procedure

The creatinine certified reference material, the isotopically labelled creatinine as well as the water samples for calibrating the 50 µl-syringe and the Hamilton pipettor are weighed with an electronically automated balance (model 4503, Sartorius, Göttingen, FRG). The volumetric flask is calibrated with the use of a mechanical balance (model 2432, Sartorius, Göttingen, FRG). The accuracy of the balances is ascertained by the use of officially calibrated test weights.

Instruments and Settings

For preparative HPLC a stainless steel column (25 × 0.8 cm, Knauer, Berlin, FRG) is packed with Lichrosorb RP-8, 5 µm (Merck, Darmstadt, FRG). Chromatography is carried out with a constant flow pump (model 600) and a UV-detector (model SP-4) which is set to 235 nm; both instruments are from Gynkotek, München, FRG. For gas liquid chromatography a gas chromatograph model 4100 (Carlo Erba, Milano, Italy) is used. The instrument is equipped with a fused silica capillary column, 30 m × 0.32 mm, coated with a film of 0.25 µm SE-52 (J & W Scientific, Rancho Cordova, USA). The temperature of the column is programmed from 140 to 180°C with a rate of 2°C/min. Nitrogen is used as carrier gas at an inlet pressure of 70 kPa. Substances are injected into a split inlet device (Gerstel, Mülheim, FRG) which is kept at 240°C. The split ratio is set to a value of about 1 : 10.

For GC-MS a combined gas chromatograph-mass spectrometer model 1020 (Finnigan MAT, Bremen, FRG) is used. The conditions for gas liquid chromatography are the same as described above with the exception that helium is used as carrier gas at an inlet pressure of 35 kPa. The end of the column is

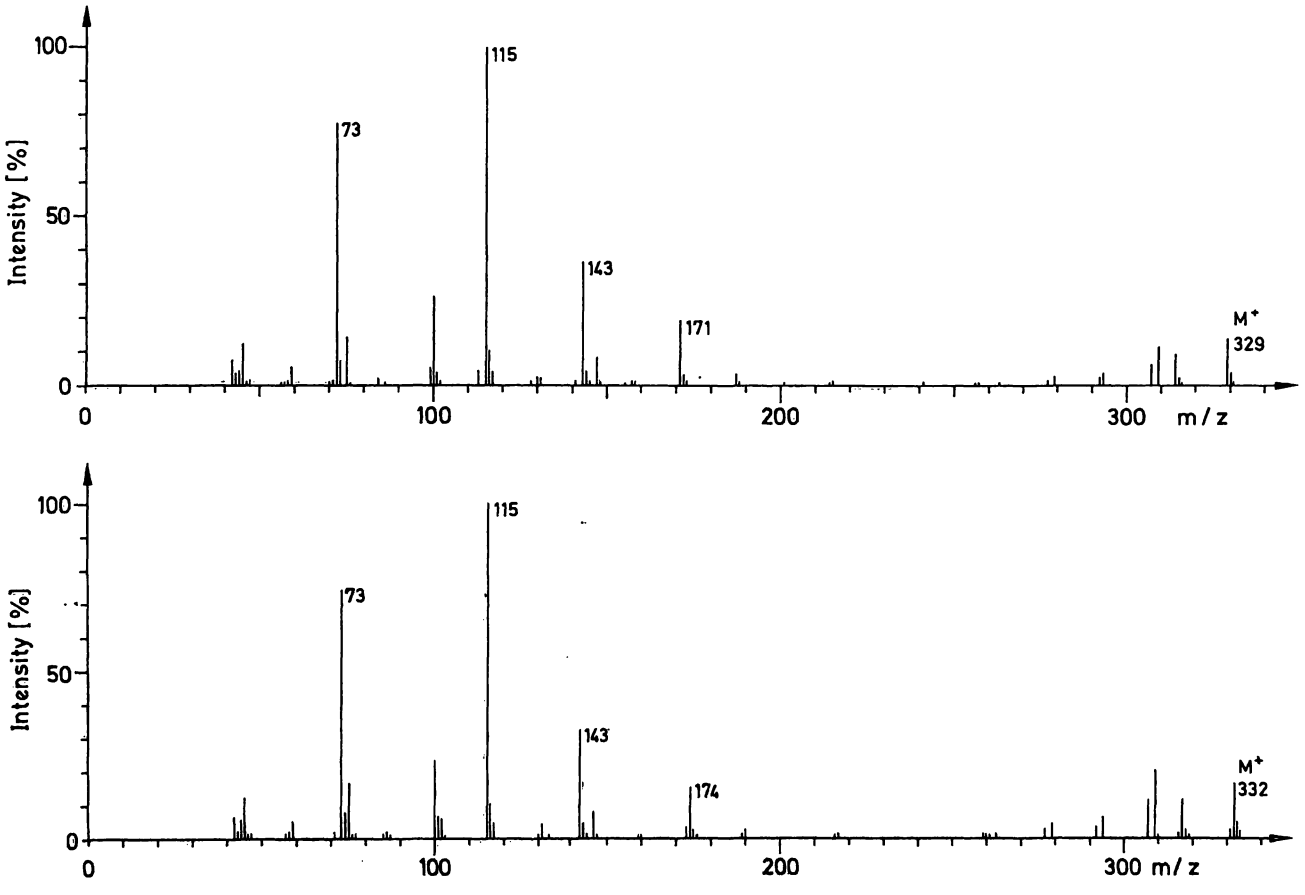

Fig. 1. Mass spectrum of the trimethylsilyl derivative of creatinine (upper panel) and of [^{13}C,$^{15}N_2$]creatinine (lower panel). For conditions of recording see Instruments and Settings.

via a divert valve to the mass spectrometer. During the first 140 s of chromatography the valve is opened in order to suck off solvents and reagents thereby protecting the ion source of the mass spectrometer from an excess of substances.

Electron impact ionisation is used for mass spectrometry. The ionisation energy is 70 eV. With the selected ion recording mode the quadrupole filter of the mass spectrometer is adjusted to a resolution of about 400 (10% valley definition). For the recording of mass spectra the instrument is scanned in the range from m/z 50 to 400; in this case the resolution is set to approximately 800.

Preparation of Standard Solutions

A specified amount of certified reference creatinine (purity 99.8%, estimated inaccuracy 0.1%) in the range of 1.45 to 1.55 mg (e.g. 1.526 mg) is dissolved in 100 ml distilled water using the calibrated volumetric flask at 20°C. The weight is corrected with respect to the impurities of the reference material by the factor 0.998 and according to the calibrated volume of the flask by multiplying by 0.99689, thus yielding a final concentration of 15.182 µg/ml. A new standard solution is prepared for each occasion.

For the preparation of the [^{13}C,$^{15}N_2$]creatinine standard solution about 3 mg of the labelled compound are dissolved in 25 ml distilled water. The solution is stored in portions of 5 ml at − 20°C. One portion, which is used as the labelled working solution, is kept in a refrigerator at 4°C; this is stable for at least 3 weeks. The material is equilibrated to 20°C prior to use.

Preparation of Standards

For the calibration of the creatinine measurement three standards with different amounts of creatinine are prepared for each batch of analyses:

Standard 1 contains a mixture of 25 µl of the labelled creatinine and 150 µl of the non-labelled creatinine solution,

standard 2 consists of 25 µl labelled and 200 µl non-labelled working solution and

standard 3 is a mixture of 25 µl labelled and 250 µl non-labelled creatinine standard solution. The labelled creatinine is pipetted with the 50 µl-syringe and the non-labelled substance is dispensed with the Hamilton Microlab P pipettor.

Each of the three standards is prepared in triplicate. The exact volumes of the non-labelled standard solution, as measured by calibrating the Hamilton pipettor, are 150.252 µl ± 0.065 µl (standard deviation, s.d.), 200.882 µl ± 0.078 µl (s.d.) and 250.976 ± 0.055 µl (s.d.), respectively. Thereby it is calculated that standard 1 contains 2.2811 µg, standard 2 3.0498 µg and standard 3 3.8103 µg of the creatinine reference material. It should be noted that it is not necessary to know accurately the amount of the labelled creatinine. However, it must be carefully checked that the standards and samples contain the same amount of the labelled substance. This is achieved by using the same syringe equipped with the repeating adaptor for the addition of the labelled substance to standards and serum samples. The standards are prepared in small tapered test tubes (total volume about 2 ml) with a ground glass stopper. The standard mixtures are evaporated to dryness in a stream of nitrogen at 80°C.

Sample Preparation

The approximate concentration of creatinine in the serum samples to be investigated is first estimated by the use of a routine method. Then aliquots of the serum containing about 3 µg creatinine are sampled with the Hamilton pipettor. A half volume (with respect to the amount of serum) of hydrochloric acid (0.1 mol/l) is added and the samples are diluted with distilled water to a final volume of 0.5 ml by the use of the electronically automated pipettor. 25 µl of the labelled creatinine solution is added and the mixture is equilibrated by gently shaking it at room temperature for 2 h. Then the diluted serum samples are transferred to the ion exchange columns. The columns are first washed with 2.5 ml distilled water in two portions (0.5 and 2.0 ml) and then with 0.75 ml 2 mol/l ammonia solution. Creatinine as well as the labelled compound are eluted by addition of a further 0.5 ml of ammonia (2 mol/l). The samples are collected in small tapered test tubes (total volume about 2 ml) with a ground glass stopper and evaporated to dryness in a stream of nitrogen at 80°C.

Derivative Formation

The dry residues of standards and samples are reacted with 30 µl of a mixture (1 : 1, by vol.) of MSTFA and pyridine (dried over molecular sieve 4 Å). The reaction is carried out at 60°C within 40 min.

Selected Ion Recording

Aliquots of about 1 µl of the reaction mixture are injected into the split inlet system and chromatographed on the SE-52 column which is coupled to the mass spectrometer. For the selected ion recording technique the quadrupole filter of the instrument is scanned over two small mass ranges which are set from − 0.25 to + 0.25 mass units from the peak center of the molecular ion of the trimethylsilyl derivative of creatinine (m/z 329) and from − 0.25 to + 0.25 mass units from the peak center of the molecular ion of the derivative of [^{13}C,$^{15}N_2$]creatinine (m/z 332).

The exact position of the peak center with reference to the mass calibration of the spectrometer is determined in a preliminary run which is regularly carried out prior to a series of quantitative measurements. The quadrupole filter of the mass spectrometer repetitively scans each of the two pre-adjusted mass ranges in 0.134 s; the complete cycle takes about 0.280 s. The intensity signals of the electron multipler of the spectrometer are recorded on magnetic discs of a dedicated computer system which is integrated in the Finnigan mass spectrometer. The selected ion chromatograms are displayed on a terminal for visual inspection of the peaks and the computer determines peak heights and areas for the measurements at the two m/z-values.

Samples and standards are injected alternately which makes it possible to monitor the stability of the instrumentation in the course of an analytical series. A selected ion recording after processing a serum sample is shown in figure 2.

Calculation Procedure

The results of the ID-MS determinations of creatinine are calculated from peak height or peak area ratios measured by the selected ion monitoring technique. Since there is a mass difference of 3 units between the non-labelled and the labelled creatinine, the contribution of naturally occurring isotopes of creatinine at m/z 332 is rather low. This results in an almost linear function between the peak height or peak area ratios and the amount of creatinine in samples and standards. Furthermore it should be noted that the labelled creatinine contains a very small amount of less than 1% non-labelled creatinine. Although these alternate isotope contributions are rather small they are taken into account by a calculation procedure which is reported in detail in the first paper of the present series on definitive methods in clinical chemistry (23).

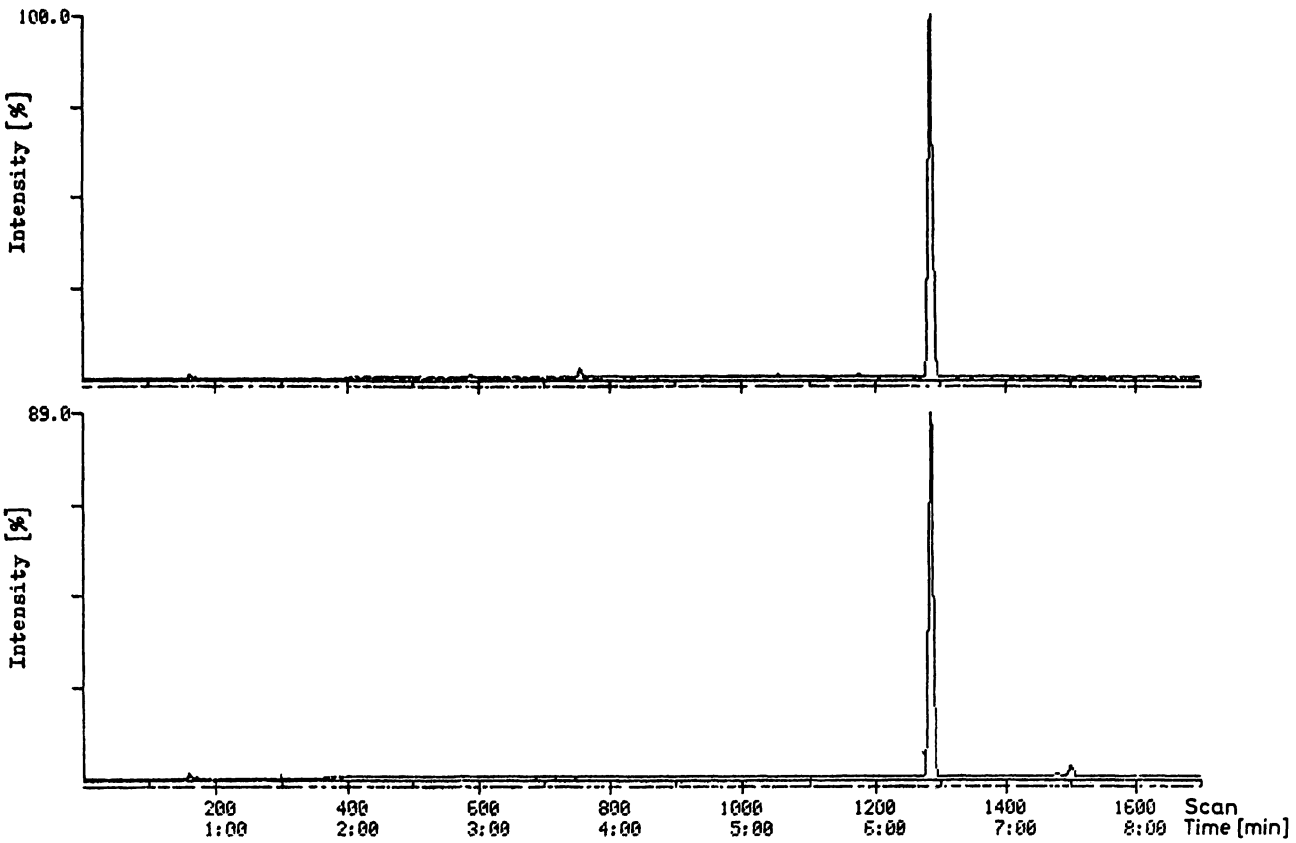

Fig. 2. Selected ion recording (computer print) of the trimethylsilyl derivative of creatinine (upper panel) and of [^{13}C,$^{15}N_2$] creatinine after processing a serum sample. For experimental details see Materials and Procedure.

Accuracy

The accuracy of the method is ascertained by the combination of two analytical principles: The isotope dilution method which is applied here is the most reliable procedure for monitoring recovery during the analytical procedure. The second methodological principle employed here is the selected ion monitoring technique. At present, this method is generally accepted as the most specific procedure for the measurement of substances in a biological matrix. Therefore, it may be assumed that the ID-MS technique, which has also been elaborated in this laboratory for the measurement of steroid hormones (23–25) and uric acid (26), may also be successfully employed for the highly accurate measurement of creatinine in human serum.

Precision

The precision of the isotope dilution-mass spectrometry measurement of creatinine in human serum is determined by repetitive measurements of the compound in several serum samples on different occasions. For this purpose lyophilised control sera which are distributed by the Deutsche Gesellschaft für Klinische Chemie for external quality control are analysed on different occasions. The lyophilised materials of 5 vials are reconstituted by addition of distilled water; then the reconstituted sera are pooled and frozen at −20°C in portions of 1 ml. For each analytical series one of these portions is thawed and equilibrated to room temperature prior to analysis. By this procedure any additional influence of a vial to vial variation between the lyophilised samples may be excluded and only the method-dependent standard deviation is obtained. As shown in table 1 the coefficient of variation is in a range from 0.32 to 1.04%.

Sensitivity

The combined gas chromatograph-mass spectrometer is capable of recording 0.5 ng creatinine as trimethylsilyl derivative injected into the split inlet of the system with a signal to noise ratio of 3:1. It should be mentioned that more than the hundredfold amount is used in the analyses of serum samples.

Tab. 1. Concentration of creatinine measured by ID-MS in control sera for external quality control and parameters of precision.

Serum pool	Creatinine (µmol/l)	n	Standard deviation (µmol/l)	Coefficient of variation (%)
321	79.92	8	0.80	1.00
320	121.11	8	1.09	0.90
380	132.60	8	0.93	0.93
319	148.52	8	1.49	1.00
379	152.94	10	1.07	0.70
378	207.75	8	2.16	1.04
322	293.50	8	2.38	0.81
381	318.25	7	1.24	0.30
323	376.60	14	2.64	0.70
382	418.14	8	1.34	0.32
324	466.77	10	3.73	0.80
383	481.79	7	2.55	0.53
281	535.72	10	4.66	0.87

Discussion

The concentration of creatinine in serum is one of the most important clinical chemical parameters for monitoring renal function. Much effort has been devoted to the development of reliable methods for the determination of the substance in clinical chemistry (1 – 20). At present, a variety of methods employing different analytical principles are in use in clinical chemical laboratories. In external quality control it is observed that, by using various methods for the analysis of creatinine in the same serum sample, a scatter of results is obtained which show more or less significant discrepancies. For this reason it was necessary to apply a variety of method-dependent target values in external quality control in order to provide a fair basis for the evaluation of the results of the participants in collaborative surveys.

It therefore seems to be necessary to develop reference and definitive methods for the measurement of creatinine in human serum, by which the accuracy of the routine methods may be critically judged. Moreover, reference and definitive methods in future may provide the most suitable basis for certifying target values for collaborative surveys, thereby replacing the currently used method-dependent assigned values.

In the present investigation a method was developed which is based on the principle of isotope dilution-mass spectrometry. It is generally accepted that this technique, at present, provides one of the most specific and accurate means for the measurement of chemical substances in a complex biological matrix. Therefore, the ID-MS technique has been used for the development of several reference and definitive methods in clinical chemistry which were reviewed by *Tietz* in 1979 (27). More recently the technique has been proposed as a reference or definitive method for the measurement of cholesterol (28), cortisol (23, 29, 30), oestradiol-17β (24, 25), uric acid (26) and thyroxine (31). *Björkhem* and coworkers (18) reported an ID-MS method for the measurement of creatinine which comprises the use of [$^{15}N_2$]creatinine as internal standard, extraction of creatinine with ethanol, purification of the samples by HPLC using gradient elution, formation of a (2-hydroxy, 2-methyl)ethyl-ditrifluoroacetate derivative in a two step reaction and finally GC-MS using a packed SE-30 column.

In the present investigation, which aspires to the development of a definitive method, the use of creatinine as a primary standard material of high purity is an imperative necessity. Fortunately, a certified reference material was available from the National Bureau of Standards, Washington, which fulfilled these requirements. With regard to the isotopically labelled creatinine there was no preparation commercially available which was suitable as internal standard for ID-MS. Therefore it was necessary to synthesize a labelled creatinine. It seemed to be advantageous to make use of a compound which differs from the non-labelled creatinine by at least 3 mass units, since the application of a material with a difference of only 2 mass units would result in a non-linear calibration curve thereby restricting the possibility of precise measurements to a small range of concentrations of creatinine in the samples. Therefore a simple preparative procedure starting from labelled cyanamide was elaborated which yields a labelled creatinine containing one ^{13}C- and two ^{15}N-atoms. This compound proved to be much more suitable for internal standardization in selected ion monitoring as compared with the double labelled substance as used by *Björkhem* et. al. (18). After equilibration of the labelled with the non-labelled creatinine the two compounds were extracted from the serum samples by the use of an ion exchange resin. By this very simple procedure, applied here, several groups of accompanying impurities from the biological matrix, e. g. proteins and lipids, were removed from the samples. Creatinine and the labelled analogue were then converted into the tris-trimethylsilyl derivative, which has already been described by *Lawson* in 1975 (32). The substance was easily prepared in a one-step-reaction. As demonstrated from the mass spectra of the creatinine derivative and that of the labelled compound in figure 1, the molecular ions at m/z 329 and 332 show fairly high relative intensities after electron impact ionisation. Therefore a sensitive detection of the two substances was possible when the

combined GC-MS instrument was run in the electron impact ionisation mode. It was not necessary to use alternative ionisation techniques such as chemical ionisation. When the reaction mixture, obtained after processing a serum sample, was injected into the instrument which was adjusted to monitor m/z 329 and 332, only two peaks corresponding to the labelled as well as the non-labelled creatinine derivative were observed, as shown in figure 2. This demonstrates the high specificity of the selected ion monitoring procedure. In addition a capillary gas chromatography column of high performance was coupled to the mass spectrometer in order to separate any compound from the biological matrix that possibly could interfere with the detection of creatinine and its labelled analogue, thereby increasing specificity and accuracy of the method. For this reason, capillary gas chromatography seemed to be advantageous as compared with other separation methods e. g. high performance liquid chromatography.

In order to calibrate the GC-MS instrument for quantitative analyses three standards containing different amounts of non-labelled creatinine were prepared, encompassing the amount of the substance in the serum samples; standards and samples were injected alternately into the GC-MS instrument. As already mentioned the contribution of naturally occurring isotopes of the creatinine molecule to the recording of the labelled creatinine is rather small when using a threefold labelled analogue.

Nevertheless, this interference as well as the presence of very small amounts of non-labelled creatinine in the labelled internal standard are taken into consideration by applying a calibration function which has been reported in detail in the first article of this series (23).

Despite all efforts that have been devoted to the development of highly accurate procedures for the use as definitive methods, there exists no final proof of accuracy in general. With the creatinine method described here there remain some very small sources of possible error. These are mainly due to the uncertainty of the creatinine content (99.8%) in the certified reference material which is less than 0.1%. A further source of possible systematic error concerns the balances and the accuracy of the calibrated test weights which are used for weighing the creatinine standard material and for the calibration of the volumetric instrumentation. The possible error due to this uncertainty is estimated to be less than 0.2%. In addition to the unknown systematic errors the final results of the analyses are affected from the imprecision (0.32 to 1.04%, coefficient of variation) which is mainly due to the instability of the isotope ratio measurement with the mass spectrometer and to the limited precision of measuring the volumes of serum samples and standard solutions.

In conclusion it may be stated that, apart from a few sources of unverifiable error, the procedure reported here is capable of producing results which are as accurate as possible with reference to the present state of technology. In view of the high specificity of the selected ion monitoring technique and due to the exact control of recovery employing the isotope dilution principle, the procedure reported here may be proposed as a definitive method for the measurement of creatinine in human serum.

The method has been used for about two years for the measurement of creatinine in the control sera which were distributed for the collaborative surveys of the Deutsche Gesellschaft für Klinische Chemie.

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